

WHAT IS CLAIMED:

1. A method of large scale virus production, which comprises:
 - a) inoculating a cell growth medium with a population of host cells,
5 wherein the cell growth medium contains an effective amount of a shear-protective compound;
 - b) culturing the host cells in the cell growth medium;
 - c) infecting the host cells in the cell growth medium with an aliquot of
10 a virus seed stock, wherein the virus seed is essentially free of any cell-lysing component;
 - d) culturing the virus infected cells of step c) under gas sparging;
 - e) harvesting intracellular and/or extracellular virus from the host cells
and cell growth medium; and,
 - f) purifying the harvested virus of step e).
- 15 2. A method of claim 1 wherein the shear-protective compound is selected from the group consisting of PLURONIC®F-68, other PLURONIC® copolymers, hydroxyethyl starch, derivatives of cellulose, serum, tryptosephosphate, polyvinyl alcohol (PVA), bovine serum albumin, polyethylene glycol (PEG), and
20 dextran.
3. The method of claim 2 wherein the shear-protective compound is PLURONIC®F-68.
- 25 4. The method of claim 3 wherein PLURONIC®F-68 is present at a concentration from about 0.3 g/L and to about 10 g/L.
5. The method of claim 4 wherein PLURONIC®F-68 is present at a concentration from about 1 g/L to about 2 g/L.
- 30 6. The method of claim 1 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.1 VVM.
7. The method of claim 6 wherein gas sparging is provided at a rate
35 corresponding to a rate up to about 0.001 to 0.05 VVM.

8. The method of claim 2 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.1 VVM.
- 5 9. The method of claim 8 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.001 to 0.05 VVM.
- 10 10. The method of claim 3 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.1 VVM.
- 11 11. The method of claim 10 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.001 to 0.05 VVM.
- 15 12. The method of claim 4 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.1 VVM.
- 20 13. The method of claim 12 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.001 to 0.05 VVM.
- 25 14. The method of claim 5 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.1 VVM.
- 30 15. The method of claim 14 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.001 to 0.05 VVM.
16. A method of large scale adenovirus production, which comprises:
- a) inoculating a cell growth medium with a population of host cells, wherein the cell growth medium contains an effective amount of a shear-protective compound;
 - b) culturing the host cells in the cell growth medium;
 - c) infecting the host cells in the cell growth medium with an aliquot of a adenovirus seed stock, wherein the adenovirus seed is essentially free of any cell-lysing component;
 - d) culturing the adenovirus infected cells of step c) under gas sparging;

- e) harvesting intracellular and/or extracellular adenovirus from the host cells and cell growth medium; and,
- f) purifying the harvested adenovirus of step e).

- 5 17. A method of claim 16 wherein the shear-protective compound is selected from the group consisting of PLURONIC®F-68, other PLURONIC® copolymers, hydroxyethyl starch, derivatives of cellulose, serum, tryptosephosphate, polyvinyl alcohol (PVA), bovine serum albumin, polyethylene glycol (PEG), and dextran.
- 10 18. The method of claim 17 wherein the shear-protective compound is PLURONIC®F-68.
- 15 19. The method of claim 18 wherein PLURONIC®F-68 is present at a concentration from about 0.3 g/L and to about 10 g/L.
20. The method of claim 19 wherein PLURONIC®F-68 is present at a concentration from about 1 g/L to about 2 g/L.
- 20 21. The method of claim 16 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.1 VVM.
22. The method of claim 21 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.001 to 0.05 VVM.
- 25 23. The method of claim 17 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.1 VVM.
24. The method of claim 23 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.001 to 0.05 VVM.
- 30 25. The method of claim 18 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.1 VVM.

26. The method of claim 25 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.001 to 0.05 VVM.

5 27. The method of claim 19 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.1 VVM.

28. The method of claim 27 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.001 to 0.05 VVM.

10 29. The method of claim 20 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.1 VVM.

30. The method of claim 29 wherein gas sparging is provided at a rate corresponding to a rate up to about 0.001 to 0.05 VVM.

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31. A method of producing a virus seed stock free of cell-lysis components, comprising:

- a) inoculating and culturing host cells in a cell growth medium free of cell lysis components;
- 20 c) infecting the host cells of step a) with a virus, resulting in virus-infected host cells;
- d) culturing the virus-infected host cells;
- e) harvesting intracellular and/or extracellular virus from the host cells and cell growth medium without the aid of any added cell-lysis component; and,
- 25 f) concentrating the harvested virus of step e), resulting in a unclarified virus seed stock.

32. The method of claim 31 wherein the viruses of step e) are released from host cells by non-mechanical shearing.

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33. The method of claim 31 wherein the viruses of step e) are released from host cells by mechanical shearing.

34. The method of claim 33 wherein the mechanical shearing options are selected from the group consisting of hollow fiber ultrafiltration, plate and frame

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microfiltration, ultrasonics, high pressure homogenization, pumps, impinging jets and mechanical grinding.

35. The method of claim 31 wherein steps e) and f) are simultaneously
5 accomplished by hollow fiber ultrafiltration of the infected cell culture for cell lysis and volume reduction.

36. An unclarified virus seed stock prepared by the method of
10 claims 31-35.

37. A method of producing a adenovirus seed stock free of cell-lysis
components, comprising:

- a) inoculating and culturing host cells in a cell growth medium free of cell lysis components;
- 15 c) infecting the host cells of step a) with a adenovirus, resulting in adenovirus-infected host cells;
- d) culturing the adenovirus-infected host cells;
- e) harvesting intracellular and/or extracellular adenovirus from the host cells and cell growth medium without the aid of any added cell-lysis component;
- 20 and,
- f) concentrating the harvested adenovirus of step e), resulting in a unclarified virus seed stock

38. The method of claim 37 wherein the viruses of step e) are released
25 from host cells by non-mechanical shearing.

39. The method of claim 37 wherein the viruses of step e) are released from host cells by mechanical shearing.

30 40. The method of claim 39 wherein the mechanical shearing options are selected from the group consisting of hollow fiber ultrafiltration, plate and frame microfiltration, ultrasonics, high pressure homogenization, pumps, impinging jets and mechanical grinding.

41. The method of claim 37 wherein steps e) and f) are simultaneously accomplished by hollow fiber ultrafiltration of the infected cell culture for cell lysis and volume reduction.

5 42. An unclarified virus seed stock prepared by the method of claims 37-41.

 43. The method of claim 31 wherein the unclarified virus seed stock is further subjected to a clarification step.

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 44. A clarified virus seed stock prepared by the method of claim 43.

 45. The method of claim 37 wherein the unclarified virus seed stock is further subjected to a clarification step.

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 46. A clarified virus seed stock prepared by the method of claim 45.